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THE FINE STRUCTURE OF THE AMPHIBIAN LYMPH HEART

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ABSTRACT

The fine structure of an amphibian lymph heart is described. There was an overlapping, flattened endothelial lining on the inner aspect of the tunica intima. This was usually thin with irregular processes on both luminal and abluminal surfaces, especially on the afferent ostium. Most of the intercellular junctions had a number of adhesion devices and were often convoluted. Very few open junctions were seen. The basement membrane was usually absent, but on occasion was prominent. The abluminal cytoplasmic projections of the endothelial lining often bordered on dense elastic and collagenous fibers. The surrounding media was thick and was mainly composed of smooth muscle cells and interstitial tissue with occasional fibroblasts.

The morphology and histological structure of the lymph heart in the amphibian was studied early in this century (1-4). The lymphatic system of the higher vertebrates has also been studied by electron microscopy (5-8), including the lymph hearts of birds (5) and the microcirculation of the more primitive vertebrates (9). However, the fine structure of the lymph heart in amphibians has not been described. In view of increasing physiological interest in this organ, it is appropriate that we now describe its ultrastructure.

MATERIALS AND METHODS

Adult Queensland cane toads (*Bufo fowleri*) were used in the study. They were pithed initially. In order to find the lymph hearts more easily, separate injections were made into the dorsal lymph sac and the toe webs of the hindlimbs of the toads, using 0.5ml and 1.0ml of China (Indian) ink (Pelikan, Günther Wagner, Hannover, Batch No. C11/1431a). They were killed 40 seconds after injection, the posterior hearts were dissected free, and transferred into 3% glutaraldehyde buffered with 0.1M phosphate at pH 7.4 for 30 minutes. The specimens were divided into ~1mm^3 pieces under a dissecting microscope, and then further fixed in the same fixative solution for 4 hours. After washing in 0.2M phosphate buffer, they were post-fixed in 1% osmium tetroxide in phosphate buffer for 60 minutes. They were then dehydrated in ascending alcohols and embedded in araldite by the usual methods. Semi-thin sections were used to find suitable regions. Nine blocks were used and 3 to 5 grids were examined from each block. Sections were stained with uranyl acetate and lead citrate (pH 10).

RESULTS

The basic ultrastructure of the tunica intima is shown in *Figs. 1-3 and 7*. The inner lining is composed of flattened elongated endothelium which frequently

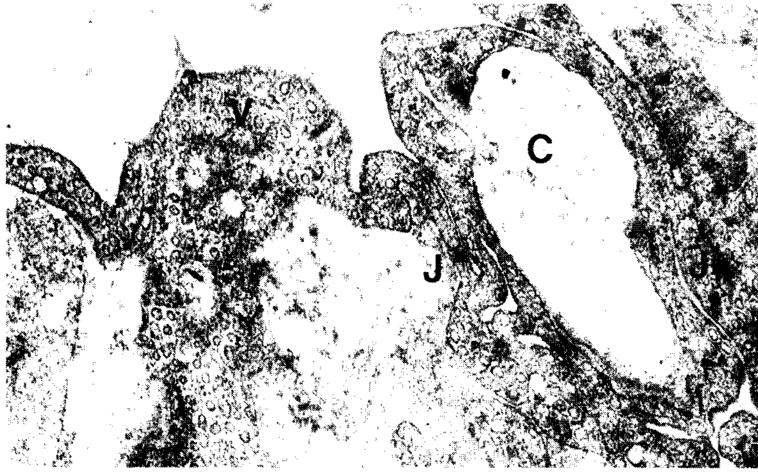

Fig. 1. Collagenous fibers (C) surround and are attached to endothelial projections. Numerous smooth vesicles (V) can be seen in the cytoplasm. The intercellular junctions (J) are complex. x25,000.

forms undulating processes extending into the lumen or sinking into the interstitial tissue from the abluminal surface *(Figs. 1-3)*. Endothelial intercellular junctions have varying degrees of complexity *(Figs. 1-5)*, often with zonulae adherentes and occludentes *(Fig. 4)*. At times they overlap and interdigitate in a most complex manner *(Fig. 2)*. There are occasional open junctions *(Fig. 5)*. The endothelial cytoplasm contains the usual organelles, including many small smooth vesicles (~50nm) and a few dense coated ones (40-160nm). At times the endothelium, with an inner core of connective tissue, is in valve-like configuration.

Fig. 2. There is a very complex junction formed by processes from three cells. Some zonulae adherentes (arrows) are seen in some areas of the junctions. x12,000.

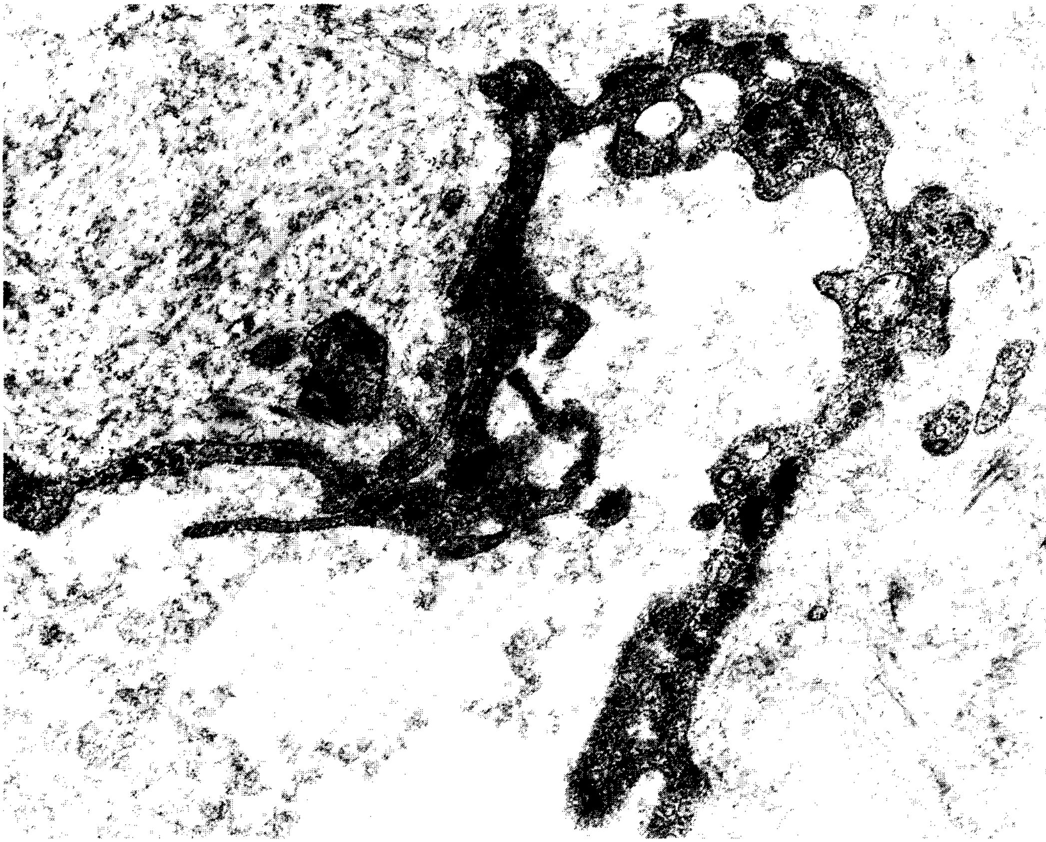

Fig. 3. There are many projections from the luminal and adluminal surfaces. The latter have anchoring filaments attached to them. A fairly simple junction is visible. x20,000.

The basement membrane was rarely visible *(Figs. 1-3,5),* but it was occasionally quite pronounced. The cytoplasmic projections into the interstitium were usually surrounded by collagen and finer fibrils *(Figs. 1-3)*. The endothelial projections were often joined to the frequent elastic fibers by fine fibrils. These fibrils also formed the usual anchoring filaments, which passed between the endothelium and the connective tissue, especially the collagen fibers *(Fig. 3)*.

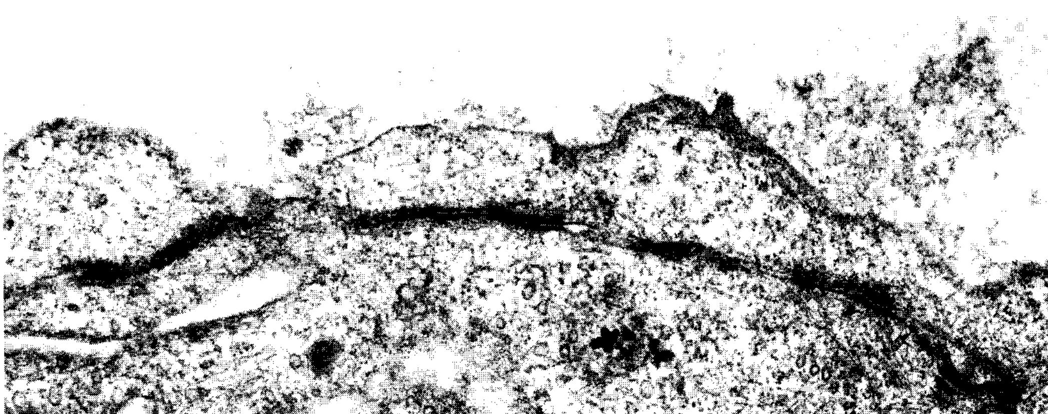

Fig. 4. There are many zonulae adherentes between two endothelial cells. x22,000.

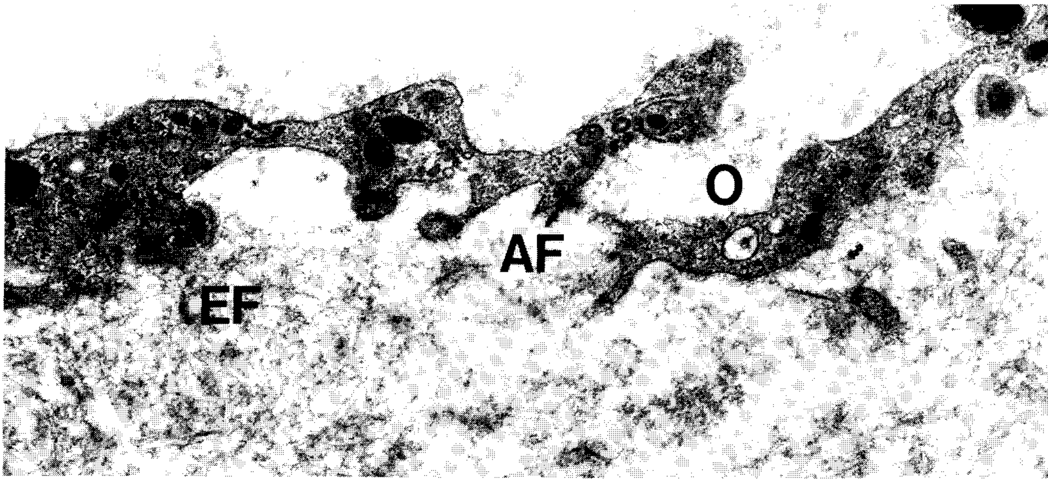

Fig. 5. An open junction (O). Elastic fibers (EF) and anchoring filaments (AF) attached tightly to the cytoplasmic projections. x15,000.

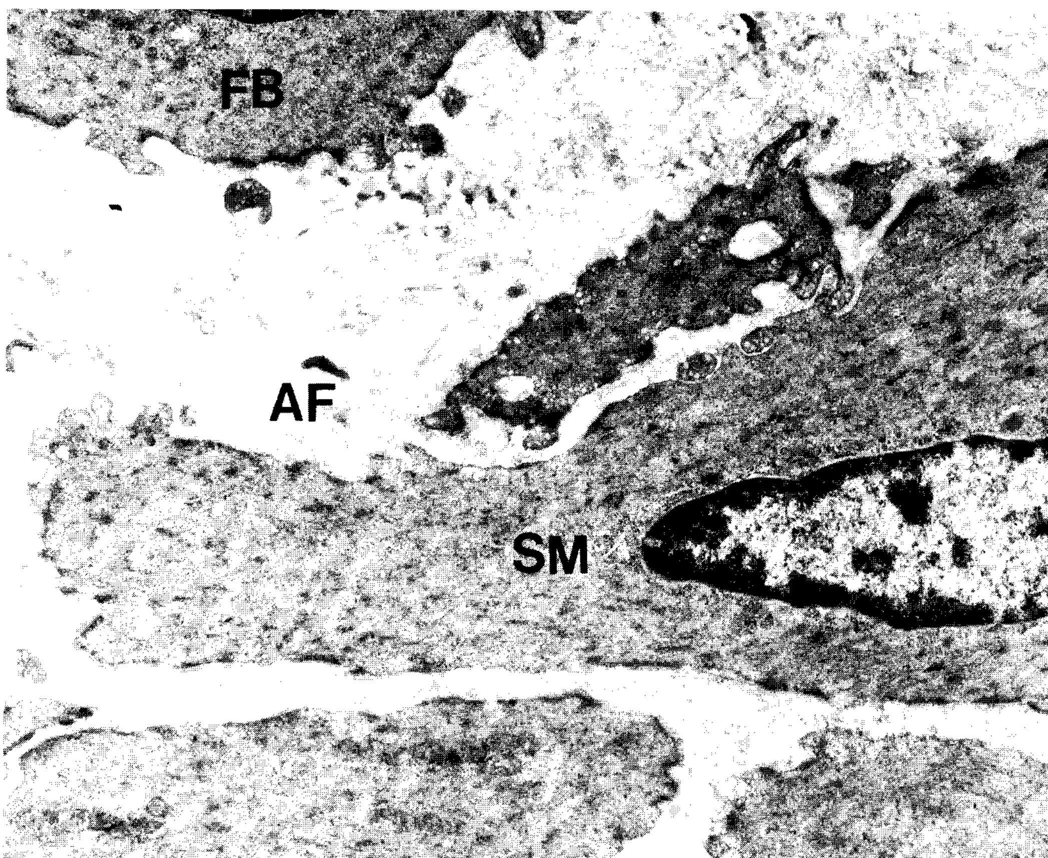

Fig. 6. Smooth muscle cells (SM) are visible in the tunica media. These are irregular. A fibroblast on the left top corner can be seen (FB) in the outer layer of the tunica intima. Filaments similar to anchoring filaments are visible between the cells and very close to them (AF). x10,000.

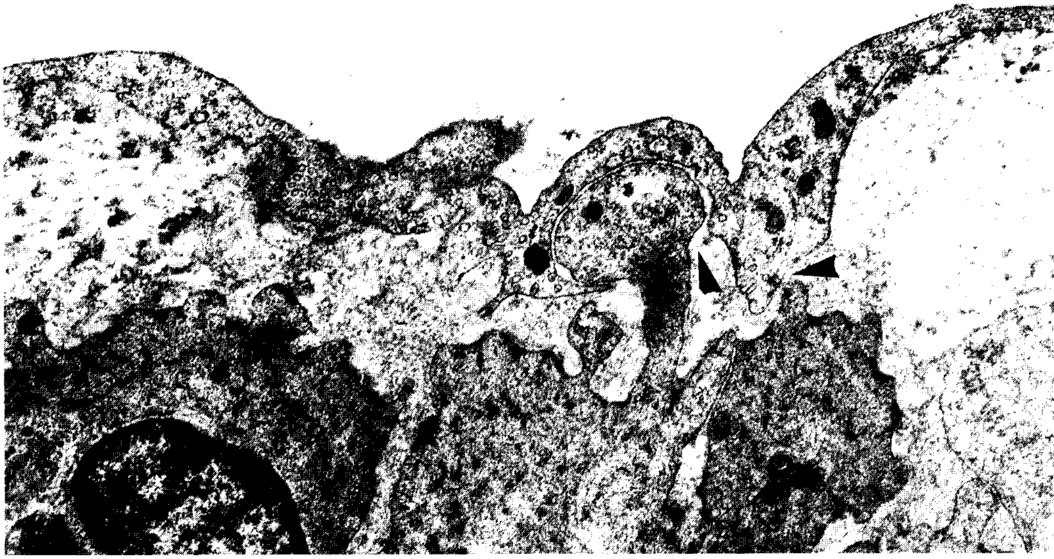

Fig. 7. A projection from a smooth muscle cell approaches very close to the endothelium (arrows). x13,000.

Depending upon the site, the tunica media was composed of smooth muscle cells and fibers of varying thickness. The outlines of the smooth muscle cells were frequently irregular. Filaments similar to anchoring filaments sometimes extended very close to their plasma membranes *(Fig. 6)*. Sometimes extensions of a smooth muscle cell closely approached the endothelium *(Fig. 7)*, suggesting the possibility of transmission of signals one way or the other. There were occasional fibroblasts and granulocytes, blood vessels and nerves in the media and adventitia. The connective tissue collagen and elastic fibers gradually became more prominent as one passed to the adventitia, which was effectively dense connective tissue forming a capsule.

DISCUSSION

The fine structure of the endothelium of the toad lymph heart is less like mammalian initial lymphatics (6-8) and more like that of collecting lymphatics (6,10,11). Open junctions are infrequent; the tight or closed junctions are usually equipped with zonulae adherentes and occludentes and are often very complex. While open junctions are found in mammalian collecting lymphatics, and even in corresponding lymph nodes, these become progressively less frequent as one passes centrally (6,10).

However, the basement membrane in the toad lymph heart is far less regular than in mammalian collecting lymphatics and resembles more closely the tenuous one of mammalian initial lymphatics (6-8). The endothelial cells are tightly attached to elastic and collagenous fibers by the anchoring filaments. This feature, while prominent in mammalian initial lymphatics, is less common in their collecting lymphatics (6,10,11). These fibers seemed to be continuous through the muscular coat with those of the adventitia layer. This observation conforms with light microscopy (3,4).

The wall is quite muscular. The smooth muscle cells were, however, always observed in the media. In this way, the frog's lymph heart differs from that of the bird with its strangely modified skeletal muscle cells (5).

In general, one may conclude that the ultrastructure of the toad's lymph heart resembles that of the mammalian collecting lymphatic but with certain features which are more reminiscent of mammalian initial lymphatics. On the basis of fine structure alone, it is not possible to determine whether the toad lymph heart also has functional similarities to mammalian collecting lymphatics.

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REFERENCES

1. Alexander, E: *Anatomy of the Frog.* Clarendon Press, Oxford (1889).
2. Goda, T: The lymph system of the toad (*Bufo Vulgaris formosus*) as modified intercellular spaces. J. Sci. Univ. Tokyo 1 (1927), 243-275.
3. Itena, NA: Physiological characteristics of the lymphatic heart muscle in the tadpoles. (Russian). Fiziol. zh. 44 (1958), 121-132.
4. Kampmeier, OF: *Evolution and Comparative Morphology of the Lymphatic System.* Charles C. Thomas, Springfield, Ill. USA (1969).
5. Rautenfeld, DB, KD Budras: TEM and SEM investigations of the lymph heart in birds. Lymphology 14 (1981), 186-190.
6. Casley-Smith, JR: The structure and functioning of the blood vessels, interstitial tissues, and lymphatics. In: *Lymphangiology*. Foldi, M, JR Casley-Smith (Eds.), Schattauer, Stuttgart (1983), 27-164.
7. Castenholz, A: Structural and functional properties of initial lymphatics in the rat tongue. Lymphology 20 (1987), 112-125.
8. Yoffey, JM, FC Courtice: *Lymphatics, lymph and lymphomyeloid complex.* Academic Press, NY & London (1970).
9. Casley-Smith, JR: The phylogeny of the fine structure of blood vessels and lymphatics: Similarities and differences. Lymphology 20 (1987), 182-188.
10. Casley-Smith, JR: The structure of normal large lymphatics, how this determines their permeabilities and their ability to transport lymph. Lymphology 2 (1969), 15-25.
11. Mislin, H, R Schipp: Structural and functional relations of the mesenteric lymph vessels. In: *Progress in Lymphology*. Rüttimann, S (Ed.), Hafner, NY (1967), 360-365.

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